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④ Method for treating mucus-containing immunoassay samples.

④ A method and kit for the preparation of mucus-containing specimens for analysis by enzyme immunoassay. The sample is treated with an oxidant prior to immunoassay, at a concentration sufficient to reduce non-specific binding of the antibody to the mucus matrix. After this treatment the antibody is contacted with the sample and bound antibody antigen detected.

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METHOD FOR TREATING MUCUS-CONTAINING IMMUNOASSAY SAMPLES

This invention relates to immunoassay for antigens in samples which include mucus.

Immunoassays can be used in medical diagnosis for determination of the presence of a specific bacterium. One form of immunoassay involves detection of a specific bacterial antigen using a labeled antibody. For example, the antibody may be labeled with detectable enzyme, such as horseradish peroxidase, so that the presence of the antibodies can be detected by providing substrate for the enzyme, and detecting conversion of the substrate to a product. An example of one such method is described in commonly-owned co-pending U.S. Patent Application Serial No. 112,366 entitled P19 GONORRHEA IMMUNOASSAY, by Rice et al., filed October 22, 1987, hereby incorporated by reference.

One problem encountered in immunoassay procedures involves inaccuracies caused by interference from other substances in the sample. For example, false positives can arise from non-specific binding between mucus in the sample and the labeled antibody. Mucus may be naturally present, for example, in urogenital specimens collected for the purpose of diagnosing venereal disease by immunoassay. In addition, mucus can physically interfere with the ability of immunoassay to detect specimen constituents, when the analyte of interest is encrypted in the sample matrix thereby is less accessible to the test kit reagents. A well-known method for the preparation of respiratory sputum samples for diagnostic testing involves the use of mucolytic agents, such as N-acetylcysteine or dithiothreitol, to reduce the viscosity of, and to better homogenize, the specimen. These agents act on mucopolysaccharides, which are the principle constituent of the mucus, by dissociating disulfide bonds. These agents, based on thiol-mediated dissociation, are generally unsuitable for immunoassays that use heme-containing enzyme labels, such as horseradish peroxides, because of their inhibitory effect on the enzyme.

We have found that it is possible to reduce interference from mucus in the sample, without unacceptable detrimental effects on the immunoassay, by subjecting the sample to oxidative conditions. Thus, the invention features a method and kit for immunoassay using an antibody to bind an antigen in a sample having a mucus matrix. The method includes the step of subjecting the mucus-containing sample to oxidative conditions prior to immunoassay. Specifically, an oxidizing agent is provided at a concentration sufficient to prevent non-specific binding of the antibody to the mucus matrix. After this treatment the sample is processed according to standard assay techniques.

In preferred embodiments, oxidation is accomplished by treatment with hydrogen peroxide or an organic peroxide; the sample is a urogenital (particularly an endocervical) specimen; and the antigen to be detected is a *Neisseria gonorrhoeae* antigen. The kit preferably includes means to collect an endocervical sample (e.g. a swab).

This invention provides a method by which the number of the false positives in an immunoassay is reduced, without unacceptable detrimental effects on binding of the antigen and antibody in the assay, or on the ability to detect the presence of low levels of antigen.

The choice of peroxide as oxidant is particularly advantageous because of its tendency to decompose, in the presence of catalase or pseudoperoxidase activity that is naturally present in urogenital samples. Such enzymatic activity arises from the presence of normal commensal microflora and constituents of blood or epithelial cells that are unavoidably present in such samples. This sample-activated decomposition of hydrogen peroxide is advantageous because oxidants, such as hydrogen peroxide, can damage the enzyme and thereby reduce label activity. However, the hydrogen peroxide level is reduced by the above-described activity during sample incubation, so relatively high levels of peroxide may be employed without the need for an additional neutralizing step before the addition of enzyme label. Stronger oxidants than peroxide, such as periodate or perchlorates, are less suitable for enzyme immunoassay, because of their deleterious effect on the enzyme label in the absence of any neutralizing agent.

This invention is particularly suitable for detection of characteristic antigens derived from *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and herpes virus.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiment thereof, and from the claims.

This invention concerns the pretreatment of a sample for enzyme immunoassay. Generally this involves mixing the specimen with a minimum volume of an oxidative mucolytic reagent, such as hydrogen peroxide, at a concentration ranging from 1-15% (w/w) for 1-5 minutes before performing a standard immunoassay.

Sexually transmitted disease (STD) specimens from female patients are generally problematic in enzyme labeled immunoassay techniques because of the high level of false positives. However, they are particularly suited to the assay procedure of this invention.

EXAMPLE: Assay for Gonococcal Antigen

All of the following operations are performed at ambient temperature (about 20° C)

- 5 1. A urogenital swab specimen was placed in a glass tube for 5 minutes with 100 ul of a 5% solution of hydrogen peroxide in citrate phosphate buffer at pH 5 (49 mM citric acid and 0.1 M dibasic sodium phosphate).
- 10 2. The swab specimen was then placed in 500 ul of assay buffer, composed of 0.45 M potassium phosphate, 0.113 M sodium chloride, 0.56% bovine serum albumin, 0.338% Triton X-100 and 0.056% thimerosal at pH 7.2. The swab was agitated in the solution at the end of the extraction. By this process, gonococcal antigen is extracted from the organism and specimen matrix into the solution.
- 15 3. After expressing liquid from the swab, the swab was discarded.
- 16 4. To the tube containing expressed sample fluid, 50 ul of antigenococcus antibody covalently linked to horseradish peroxidase (by the method of Wilson and Nakane, in Immunoassay and Related Staining Techniques, Knapp et al., eds., 1978, pp. 215-224) is added. The tube was agitated to mix the reagents thoroughly.
- 20 5. A dipstick coated with anti-gonococcus antibody (by the method of Catt and Treager, Science 158:1570 (1967)), was then added to the tube for 30 minutes.
- 25 6. The dipstick was washed thoroughly under a stream of tap water for 5 seconds, then shaken to remove excess water.
- 26 7. The washed dipstick was then placed in a new glass tube containing 237 ul of a fresh chromogenic developer, composed of 3 parts 5.2 mM 3,3',5,5'-tetramethylbenzidine in methanol to 4 parts of 0.03% hydrogen peroxide in 49 mM citric acid, 0.1 M dibasic sodium phosphate, 0.006% sodium saponate and 0.005% thimerosal at pH 5. See, Gerber et al. U.S. Pat. 4,503,143.
- 27 8. After a 10 minute incubation, the dipstick was removed from the developer. The intensity of blue color in the developer is proportional to the quantity of gonococcal antigen present in the sample. The absence of blue indicates the absence of gonococcal antigen.

Results from the above assay were compared with that from standard culture techniques. Addition of the hydrogen peroxide treatment step increased both the sensitivity and specificity of the assay. The sensitivity is defined as the number of positives detected by the immunoassay techniques versus the number of positives detected by culture techniques; and specificity is defined as the ratio of number of negatives detected by the immunoassay techniques compared to the number detected by the culture techniques.

In particular, matched pairs of specimens were collected from individual patients. In each case individual swabs were characterized by first streaking a standard growth medium to obtain a reference result for gonococcal culture. One member of the pair was subjected to the above-described peroxide treatment, and the other was processed by standard immunoassay protocol.

The peroxide treatment improves agreement between enzyme immunoassay and culture for both positive and negative samples. Clinical sensitivity was not deleteriously affected, and specificity was enhanced.

In one embodiment, conducted generally as described above, agreement between enzyme immunoassay (EIA) and culture was improved for both positive samples (sensitivity) and negative samples (specificity).

	Sensitivity	Specificity	
	EIA+/culture+	EIA-/culture-	EIA-/culture+
+perox	9/11 = 82%	51/55	93%
-perox	6/11 = 55%	48/55	87%

Another benefit of peroxide treatment is reduction of background enzyme activity, and a resulting improvement in clinical specificity. In particular, the background level of enzyme activity in the immunoassay was measured for matched pairs of culture-negative samples. Each member of the pair was treated with extended incubation times to accentuate signal levels, either with peroxide or without peroxide and then read visually and quantitated by spectrophotometry. Peroxide treatment substantially reduced the background signal in culture-negative samples. Results are tabulated below:

	-perox	+perox
EIA-/cult-	24/38 (63%)	36/38 (96%)
Mean OD	0.10	0.02

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The target antigen is substantially unaffected by the oxidative conditions produced by the peroxide treatment. When standard laboratory preparations of gonococcal organism suspensions were assayed in the immunoassay, the effect of hydrogen peroxide pretreatment (100 microliters at 5% for 5 minutes) did not significantly affect signal intensity.

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Other embodiments are within the following claims. For example other peroxides are also useful in this invention, such as organic peroxides, including methyl hydroperoxide, t-butyl hydroperoxide, cumene hydroperoxide.

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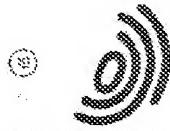
Claims

1. A method of immunoassay using an antibody to bind to an antigen in a sample comprising a mucus matrix, said method comprising the steps of:
first subjecting said mucus containing sample to oxidizing conditions prior to said immunoassay, and then contacting said antibody with said sample and detecting antibody-antigen binding in said sample.
2. A method according to claim 1 wherein said sample is treated with an oxidant, present at the concentration sufficient to reduce non-specific binding of said antibody to said mucus matrix.
3. A method according to claim 1 wherein sample mucus is oxidized with hydrogen peroxide or an organic peroxide.
4. A method according to any one of the preceding claims wherein said sample is a urogenital sample.
5. A method according to any one of the preceding claims wherein said antigen is a Neisseria gonorrhoeae antigen.
6. A method according to claim 5 wherein the antigen is H-8 antigen or P-19 antigen.
7. A method according to any one of claims 1 and 3 to 6 in which the sample comprises microflora or other constituents exhibiting pseudoperoxidase activity.
8. A kit for an immunoassay using an antibody to bind an antigen in a sample comprising a mucus matrix, said kit comprising:
an oxidant, and
an antibody labelled with a detachable label.
9. A kit according to claim 8 wherein said oxidant is hydrogen peroxide or an organic peroxide.
10. A kit according to either of claims 8 and 9 wherein said kit comprises means to collect a urogenital sample.
11. A kit according to any one of claims 8 to 10 wherein said antibody is one that specifically binds a Neisseria gonorrhoeae antigen.
12. A kit according to claim 11 wherein said antigen is H-8 antigen or P-19 antigen.

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(8) Method for treating mucus-containing immunoassay samples.

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EUROPEAN SEARCH
REPORT

Application Number

EP 89 30 6211

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
A	WO-A-8 605 592 (NATIONAL RESEARCH DEVELOPMENT CORP.) * Claim 1 * - - -	5,6,11,12	G 01 N 33/53 G 01 N 33/571
A	US-A-4 497 900 (ABRAM et al.) * Abstract * - - -	5,11	
A	EP-A-0 103 958 (GYVA CO.) * Pages 1,2 * - - -	1	
A	EP-A-0 141 244 (TERUMO K.K.) * Abstract; pages 14,15 * - - -	1,7	
A	EP-A-0 250 691 (BOEHRINGER MANNHEIM GmbH) * Whole document * - - -	1	
A	US-A-4 703 001 (VODIAN) * Column 4, line 60 - column 5, line 19 * - - - - -	1	

TECHNICAL FIELDS
SEARCHED (Int. Cl. 5)

G 01 N
C 12 Q

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	05 March 91	OSBORNE H.H.
CATEGORY OF CITED DOCUMENTS		
X:	particularly relevant if taken alone	E: earlier patent document, but published on, or after the filing date
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A:	technological background	L: document cited for other reasons
O:	non-written disclosure	&: member of the same patent family, corresponding document
P:	intermediate document	
T:	theory or principle underlying the invention	